SEQUENCE LISTING

| <110> Noteborn, Mathieu Rohn, Jennifer Leigh Mumberg, Dominik Donner, Peter |
|---|
| <120> Modifications of Apoptin |
| <130> 2906-4996.1 |
| <140> To be assigned <141> 2001-10-19 |
| <150> US 60/242,397 <151> 2000-10-20 |
| <160> 20 |
| <170> PatentIn version 3.1 |
| <210> 1 <211> 121 <212> PRT <213> Chicken anemia virus |
| <220> <221> MISC_FEATURE <222> (1)(121) |
| <223> Apoptin (a small protein derived from chicken anemia virus) encoded by pCMV-Vp3 and by GFP-Apoptin constructs |
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| Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15 |
| Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30 |
| Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly |

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro 105 110 Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 <210> 2 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> Apoptin protein encoded by pIRESneo alanine mutants <220> <221> MISC_FEATURE <223> Differs from Apoptin protein encoded by pCMV-Vp3 and by GFP-Apoptin constructs by replacement of the arginine residue at position 116 with a lysine residue <400> 2 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 10 15 5 Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

| Cys Ala Asn Al 50 | la Arg Ala Pro T 55 | hr Leu Arg Se | r Ala Thr Ala 60 | Asp Asn |
|--|-------------------------|-------------------------|----------------------|-----------------------|
| Ser Glu Ser Thi | r Gly Phe Lys As 70 | sn Val Pro Asp 75 | Leu Arg Thi | Asp Gln 80 |
| Pro Lys Pro Pro | Ser Lys Lys Ar 85 | g Ser Cys Asp 90 | | Tyr Arg 95 |
| Val Ser Glu Lei 100 | u Lys Glu Ser Le 0 | eu Ile Thr Thr ' 105 | Thr Pro Ser A | arg Pro |
| Arg Thr Ala Ly | rs Arg Arg Ile Ar 12 | _ | | |
| <210> 3 <211> 121 <212> PRT <213> Chicker | n anemia virus | | | |
| <220> <221> MUTA(<222> (1)(12 <223> mutant | 1) | lanine linker-s | canning muta | ant series of Apoptin |
| <400> 3 | | | | |
| Met Asn Ala La 1 | eu Gln Glu Asp' 5 | Thr Pro Pro G | ly Pro Ser Th | r Val Phe 15 |
| Arg Pro Pro Th | r Ser Ser Arg Pro | o Leu Glu Thr 25 | Pro His Cys 30 | Arg Glu |
| Ile Arg Ile Gly | Ile Ala Gly Ile T 40 | hr Ile Thr Leu | Ser Leu Cys 45 | Gly |
| Cys Ala Asn Al | la Arg Ala Pro T 55 | hr Leu Arg Se | er Ala Thr Ala 60 | a Asp Asn |
| Ser Glu Ser Th | r Gly Phe Lys As | sn Val Pro Asp 75 | Leu Arg Th | r Asp Gln 80 |

Pro Lys Pro Pro Ser Ala Ala Ala Ala Ala Asp Pro Ser Glu Tyr Arg 95 85 Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro 105 Arg Thr Ala Arg Arg Ile Arg Leu 115 <210> 4 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> mutant Ala(5)-91 of 5-alanine linker-scanning mutant series of Apoptin <400> 4 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 10 Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 40 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 55 60 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Ala Ala Ala Ala Ala Arg 85 90 Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 <210> 5 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> mutant Ala(5)-96 of 5-alanine linker-scanning mutant series of Apoptin <400> 5 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 40 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 55 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Ala 90 85 Ala Ala Ala Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro 100 105 110 Arg Thr Ala Arg Arg Ile Arg Leu

120

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120

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<221> MUTAGEN
<222> (1)..(121)
<223> mutant Ala(5)-106 of 5-alanine linker-scanning mutant series of Apoptin
<400> 7
Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
                                    10
Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
                              25
Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
                        40
Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
                        55
Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
                   70
Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
                                  90
Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Ala Ala Ala Arg Pro
           100
                              105
                                                 110
Arg Thr Ala Arg Arg Arg Ile Arg Leu
        115
                           120
<210> 8
<211> 121
<212> PRT
<213> Chicken anemia virus
<220>
<221> MUTAGEN
<222> (1)..(121)
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<223> mutant Ala(5)-111 of 5-alanine linker-scanning mutant series of Apoptin

| <400> 8 |
|--|
| Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15 |
| Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30 |
| Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45 |
| Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60 |
| Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80 |
| Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95 |
| Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Pro Ser Ala Ala 100 105 110 |
| Ala Ala Arg Arg Ile Arg Leu 115 120 |
| <210> 9 <211> 121 <212> PRT <213> Chicken anemia virus |
| <220> <221> MUTAGEN <222> (1)(121) <223> mutant Ala(5)-116 of 5-alanine linker-scanning mutant series of Apoptin |

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

<400> 9

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

| Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45 |
|--|
| Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asr 50 55 60 |
| Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Glr 65 70 75 80 |
| Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95 |
| Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro 100 105 110 |
| Arg Thr Ala Ala Ala Ala Ala Leu 115 120 |
| <210> 10 <211> 121 <212> PRT <213> Chicken anemia virus |
| <220> <221> MUTAGEN <222> (1)(121) <223> single point mutant T106A of Apoptin |
| <400> 10 |
| Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15 |
| Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30 |
| Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45 |
| Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60 |

| Ser Glu Ser Thr 65 | Gly Phe Lys 70 | | Asp Leu Arg T 75 | hr Asp Gln 80 |
|---|-----------------------|---------------------|------------------------|-------------------|
| Pro Lys Pro Pro | Ser Lys Lys 85 | Arg Ser Cys 2 90 | Asp Pro Ser Gl | u Tyr Arg 95 |
| Val Ser Glu Let 100 | • | Leu Ile Ala 7 | Γhr Thr Pro Ser 110 | |
| Arg Thr Ala Ar 115 | g Arg Arg Ile | Arg Leu 120 | | |
| <210> 11 <211> 121 <212> PRT <213> Chicker | n anemia virus | S | | |
| <220> <221> MUTA(<222> (1)(12 <223> single p | 1) | 107A of Apo | pptin | |
| <400> 11 | | | | |
| Met Asn Ala Le 1 | eu Gln Glu As 5 | sp Thr Pro Pr 10 | • | Thr Val Phe 15 |
| Arg Pro Pro Th | _ | Pro Leu Glu 25 | Thr Pro His Cy | ~ |
| Ile Arg Ile Gly 35 | lle Ala Gly Ile 40 | | Leu Ser Leu Cy 45 | s Gly |
| Cys Ala Asn Al | a Arg Ala Pro 55 | Thr Leu Arg | g Ser Ala Thr A | Ala Asp Asn |
| Ser Glu Ser Thi | Gly Phe Lys 70 | Asn Val Pro | Asp Leu Arg 7 | Chr Asp Gln 80 |
| Pro Lys Pro Pro | Ser Lys Lys 85 | Arg Ser Cys | Asp Pro Ser Gl | u Tyr Arg 95 |

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Thr Pro Ser Arg Pro 105 Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 <210> 12 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> single point mutant T108A of Apoptin <400> 12 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 10 15 1 5 Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 45 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 95 Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Ala Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu

120

<210> 13 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> single point mutant P109A of Apoptin <400> 13 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 40 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 55 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Ala Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Ile Arg Leu 115 120 <210> 14
<211> 121
<212> PRT
<213> Chicken anemia virus
<220>
<221> MUTAGEN
<222> (1)..(121)
<223> single point mutant T106E of Apoptin
<400> 14

Met Asp Ala Lay Cla Clay Asp The Pro Pro Clay

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Glu Thr Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120

<210> 15 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> single point mutant T107E of Apoptin <400> 15 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 40 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 55 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Glu Thr Pro Ser Arg Pro

105

120

Arg Thr Ala Arg Arg Ile Arg Leu

115

<210> 16

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

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<222> (1)..(121)

<223> single point mutant T108E of Apoptin

<400> 16

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Glu Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Ile Arg Leu 115 120 <210> 17 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> double point mutation T106A107A of Apoptin <400> 17 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 40 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 55 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75 Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Thr Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120 <210> 18

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T107A108A of Apoptin

<400> 18

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe 1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Ala Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu 115 120 <210> 19 <211> 121 <212> PRT <213> Chicken anemia virus <220> <221> MUTAGEN <222> (1)..(121) <223> double point mutant T106A108A of Apoptin <400> 19 Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu 25 Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly 40 Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn 55 Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln 70 75

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg 85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Ala Pro Ser Arg Pro 100 105 110

Arg Thr Ala Arg Arg Ile Arg Leu 115 120

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<210> 20
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<212> PRT
<213> Chicken anemia virus

<220>
<221> MISC_FEATURE
<223> amino acid sequence encoding the SV40-Large T nuclear localization signal

<400> 20

Pro Pro Lys Lys Lys Arg Lys Val

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